AD_____
(Leave blank)

Award Number: W81XWH-09-1-0633

TITLE: Myeloperoxidase in the Progression of Breast Cancer

PRINCIPAL INVESTIGATOR: Barbara M. Mueller, Ph.D.

CONTRACTING ORGANIZATION:

Torrey Pines Institute for Molecular Studies
San Diego, CA 92121-1122

REPORT DATE: September 2010

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: (Check one)

X Approved for public release; distribution unlimited

Distribution limited to U.S. Government agencies only; report contains proprietary information

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
01-09-2010	Annual	28 AUG 2009 - 27 AUG 2010

4. TITLE AND SUBTITLE

Myeloperoxidase in the Progression of Breast Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-09-1-0633

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Barbara M. Mueller, Ph.D.

Email: bmueller@tpims.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Torrey Pines Institute for
Molecular Studies
3550 General Atomics CT 2-129
San Diego, CA 92121-1122

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
USAMRMC
Fort Detrick, Maryland 21702-

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The main objective of this project is to establish whether myeloperoxidase (MPO) is protective in breast cancer. We will test this concept in mouse models of human breast cancer and predict that MPO will slow tumor growth, progression and metastasis. If MPO is protective, a likely mechanism is its role in macrophage-mediated killing of breast cancer cells. This idea will be also tested in this project. There are three Specific Aims: (1) To assess mammary tumor progression in polyoma middle T oncogene (PyMT) mice expressing human MPO (huMPO) or being mouse MPO (moMPO) deficient. (2) To determine the role of huMPO in the growth and metastasis of human breast tumors in mice. (3) To test whether huMPO produced by macrophages kills breast cancer cells.

In this first year of funding we have set up the mouse colonies and breeder and are producing mice for the experiments. All methodologies, including immuno-histochemistry, PCR and qPCR detection of huMPO in mouse tissues have been established. Production of cell lines for further experiments is in progress. Initial results suggest that whereas tumor onset is not delayed in PyMT mice expressing huMPO compared to PyMT wt mice, tumor growth is attenuated. At the same time it appears that metastasis is not inhibited. These observations need to be confirmed using larger numbers of mice.

15. SUBJECT TERMS
Breast Cancer, Animal Model, Transgenic Mice, Myeloperoxidase, Tumor progression, Metastasis

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	6	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

INTRODUCTION:
The main objective of this project is to establish whether myeloperoxidase (MPO) is protective in breast cancer. We will test this concept in mouse models of human breast cancer and predict that MPO will slow tumor growth, progression and metastasis. If MPO is protective, a likely mechanism is its role in macrophage-mediated killing of breast cancer cells. This idea will be also tested in this project. There are three Specific Aims: (1) To assess mammary tumor progression in polyoma middle T oncogene (PyMT) mice expressing human MPO (huMPO) or being mouse MPO (moMPO) deficient. (2) To determine the role of huMPO in the growth and metastasis of human breast tumors in mice. (3) To test whether huMPO produced by macrophages kills breast cancer cells.

BODY:
Following is a description of the research accomplishments associated with the four tasks outlined in the approved Statement of Work.

Task #1: Generate the mouse strains required for this project by crossbreeding
Note: all strains are available in the C57Bl/6 background

The objective of this task is to generate defined breeding colonies for the tumor experiments in tasks #2 and #3. We had proposed to breed the huMPO transgene and the PyMT transgene into moMPO$^{-/-}$ mice to obtain huMPO/moMPO$^{-/-}$, PyMT/moMPO$^{-/-}$ and PyMT/wt moMPO mice. Unfortunately it became clear, that our colony of moMPO deficient mice is no longer in a pure C57Bl/6 background and thus not suitable for crossing with the other transgenics. While the moMPO deficient mice are being backcrossed, we decided to go ahead and crossed PyMT male mice with female mice having the huMPO G allele (huMPO-G) to produce cohorts for tumor assessment. We reasoned that these cohorts will be informative, because a) our preliminary data (presented in the grant proposal) showed that PyMT mammary tumors do not express moMPO and because moMPO is known not to be expressed in monocytic cells, including macrophages. Note that huMPO-G is the version of the MPO gene promotor associated with high expression and appears to be protective in women with breast cancer (Ambrosone, 2009).

We had also proposed to breed huMPO/moMPO$^{-/-}$ and moMPO$^{-/-}$ mice with immune deficient Rag2 to obtain Rag2/huMPO/moMPO$^{-/-}$ and Rag2/moMPO$^{-/-}$ mice. We have postphoned this breeding until moMPO$^{-/-}$ mice in the C57Bl/6 background are available.

Task #2: Determine the incidence, growth, progression and metastasis of PyMT mouse mammary tumors in mice expressing the human MPO gene or lacking MPO expression

The objective of this task is to evaluate whether huMPO expression is protective in mammary tumor progression in the PyMT model. We are currently producing female PyMT/huMPO-G and PyMT wt mice for evaluation of mammary tumor incidence, growth, progression and metastasis. Based on the mice available thus far it appears that all female mice develop multi-focal mammary tumors at approximately the same time; mean onset of palpable tumor is 12.6 weeks for PyMT wt (n=10) and 13.1 weeks for PyMT/huMPO-G (n=15).

Mammary tumor growth, however, appears to be delayed in PyMT/huMPO-G mice compared to PyMT wt mice. **Figure 1** summarizes the tumor volumes for all mice that have been analyzed thus far and show a trend toward reduced tumor growth. When the mice were killed 14 weeks after tumor onset or when the tumors exceeded IACUC approved

tumor volumes, tissues including mammary tumors and lungs were harvested. Initial histological analyses of mammary tumors show that in PyMT/huMPO-G mice huMPO was strongly expressed in mosaic fashion in a subset of mammary tumors cells and possibly other cell types in the tumor micro-environment. The example in **Figure 2A-C** shows loss of defined acinar structure, suggestive of progressive carcinoma stage. In an early tumor, huMPO expression was most pronounced in tumor cells at the periphery of the tumor (**Fig. 2D**) with less MPO in more advanced carcinoma cells in the center. No huMPO staining was observed in tumors from PyMT wt mice.

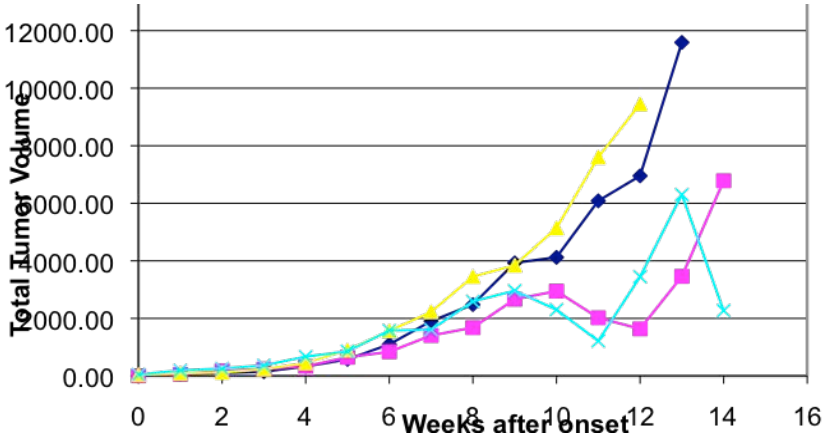

Figure 1 Mammary tumor burden of PyMT and PyMT/huMPO-G mice Mice were examined weekly for onset of tumor development. Once tumors were measurable, tumor volumes were determined by measuring the length (a) and width (b) of each tumor with calipers and calculating the volume as $a^2 \times b/2$. All tumors per mouse were added to obtain the total tumor volume per mouse. Shown are means of total tumor volumes for 10 PyMT mice (yellow and blue) and 13 PyMT/huMPO-G mice (turquoise and magenta), plotted as onset of palpable tumors (yellow and turquoise) or on set of measurable tumors (blue and magenta).

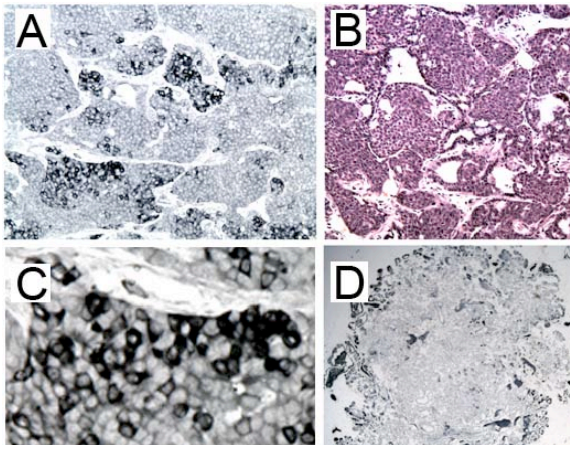

Figure 2. HuMPO immunostaining of mammary tumors from PyMT/huMPO-G mice. (A) 16 week tumor shows mosaic huMPO expression (10x obj), with mosaic expression apparent at higher magnification (C). (B) H&E staining of adjacent section to that in panel A. Panel D shows low power image of small tumor at 11 wks showing stronger huMPO staining at periphery.

Lungs of all mice were harvested and processed for histology or qPCR. Initial analysis of lungs of a small number of mice shows numerous metastatic foci in the lungs of all mice with no trend toward fewer or smaller metastases in PyMT/huMPO-G mice compared to PyMT wt mice. This observation is somewhat surprising in view of the reduced tumor growth and needs to be confirmed in a larger number of mice.

Task #3: Determine the growth and metastasis of transplanted human breast tumors in immune deficient mice that express the human MPO gene

These experiments have not yet been initiated.

Task #4: Determine whether MPO produced by macrophages kills breast cancer cells

The objective of this task is to provide evidence for our hypothesis that the protective role of MPO in breast cancer is a function of macrophage-mediated cytotoxicity.

We have determined the cytotoxicity of HOCl and H_2O_2 for human MCF-7 and MDA-MB-231 breast cancer cell lines using the MTS proliferation assays *in vitro* and found little toxicity of a wide range of concentrations. Apopotosis assays are in progress. Establishment of cell lines from advanced mammary tumors of PyMT/huMPO-G and PyMT wt mice is also in progress.

KEY RESEARCH ACCOMPLISHMENTS:

In this first year of funding we have mainly worked on setting up the mouse colonies for production of mice for the experiments. All methodologies, including immuno-histochemistry, PCR and qPCR detection of huMPO in mouse tissues have been established. Production of cell lines for further experiments is in progress.

REPORTABLE OUTCOMES:

None as of July 2010

CONCLUSION:

Initial results suggest that whereas tumor onset is not delayed in PyMT mice expressing huMPO compared to PyMT wt mice, tumor growth is attenuated. At the same time it appears that metastasis is not inhibited. These are interesting observations that need to be confirmed using larger numbers of mice.

An unexpected finding is the high expression of huMPO in what appears to be a subset of tumors cells themselves. This observation is surprising as MPO expression was thought to be restricted to cells of the hematopoietic lineages. This finding has prompted us to more closely examine human breast cancer tissues.

REFERENCES:

Ambrosone CB, Barlow WE, Reynold W eta l. Myeloperoxidase genotypes and enhanced efficacy of chemotherapy for early stage breast cancer in SWOG-8897. J Clin Oncol, 27(30):4973-9, 2009

APPENDICES:

None